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International application number: PCT/US05/011602

International filing date: 07 April 2005 (07.04.2005)

Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 60/560,307
Filing date: 07 April 2004 (07.04.2004)

Date of receipt at the International Bureau: 12 May 2005 (12.05.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

1317234

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APPLICATION NUMBER: *60/560,307*
FILING DATE: *April 07, 2004*
RELATED PCT APPLICATION NUMBER: *PCT/US05/11602*

Certified by

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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. EL 890277091 US

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Additional inventors are being named on the _____ separately numbered sheets attached hereto

TITLE OF THE INVENTION (500 characters max)

Apparatus and Method for Performing Complete Blood Counts

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

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ENCLOSED APPLICATION PARTS *(check all that apply)*

☑ Specification *Number of Pages* 10 ☐ CD(s), Number _____

☑ Drawing(s) *Number of Sheets* 3 ☐ Other (specify) _____

☑ Application Data Sheet. See 37 CFR 1.76

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☑ Applicant claims small entity status. See 37 CFR 1.27.

☑ A check or money order is enclosed to cover the filing fees.

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☐ Payment by credit card. Form PTO-2038 is attached.

FILING FEE Amount ($): 80.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☑ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are: _____

Respectfully submitted,

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Date April 7, 2004

REGISTRATION NO. 36,147 *(if appropriate)*

Docket Number: 5169-0016

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Apparatus and Method for Performing Complete Blood Counts

[0001] The complete blood count (CBC) is the most frequently performed set of tests for whole blood. The methods used vary in completeness of analyte set, complexity and cost of equipment, and per-test cost. The least complex methods, such as the QBC® method described in U.S. Patent No. 4,156,570, have the least expensive capital costs and are simple to perform but have the highest per-test costs. This method is most suited for point-of-care situations where operator training is minimal and few tests are performed per day. On the other end of the spectrum, CBC analyzers used in hospitals or reference laboratories can have a capital cost twenty times greater but a per-test cost commensurately reduced, which makes them much more cost-effective in those settings.

[0002] These larger systems, such as the Abbot Cell-Dyn® or the Bayer Advia® are based upon some variation of a flow-cytometer (FC), where a precise quantity of blood is precisely diluted and mixed with reagents in a number of steps, and where fluidic valves route the diluted sample into multiple test areas. This instrumental complexity means that the reliability of these instruments is very much lower than that for the point-of-care systems. In fact, with these larger systems it is not uncommon for preventative maintenance or repairs to be required on a weekly basis, or more often, which requires the skills of specially trained laboratory technologists or service technicians, all of which substantially add to the cost of operation. Another hidden cost of operation is the washing, cleaning and calibration procedures which are required to make the system perform properly.

[0003] Another problem with FC systems is that the samples are not "examined", in the sense that there is never any visually useful information obtained on single cells. This means that in samples where FC patterns suggest that abnormal cells may be present, the technologist must prepare, stain and examine a separate blood film to determine the cell type. This is very time consuming (and expensive), because of the frequency of these findings, the amount of labor required for these non-instrumental analyses far exceeds that for the instrument operation.

[0004] An intermediate system has been described in U.S. Patent Publication No. 20020055178, wherein blood is placed into a single-use disposable for analysis. This method can provide the same breadth of analytic data as the flow-cytometric systems

in a reliable, low-cost, and easy-to-use instrument, but the cost of the single-use disposable likely makes it unsuitable for routine use in a large laboratory setting where per-test cost is paramount.

[0005] It is the object of this invention to provide an apparatus and method for performing complete blood counts which has most or all of the following characteristics:

[0006] 1. At least the number of parameters as are available from flow-cytometric instruments.

[0007] 2. No liquid reagents or fluidics are used.

[0008] 3. No washing, adjustment or calibration is required.

[0009] 4. Per-test cost comparable to FC systems.

[0010] 5. The technologist can visually examine suspect cells so as to obviate the need for separate blood film preparation is most cases.

[0011] The present invention takes advantages of the flow characteristics of blood when spreading in a thin film while confined in a "virtual disposable", which is a single-use, disposable analysis chamber fabricated from a continuous reel of material. The reel contains sufficient material for the performance of hundreds, or thousands of individual analyses and can be inexpensively manufactured, thus keeping the per-test cost at or below that of bulk liquid reagents.

[0012] All previously invented hematology analysis systems that examine only a tiny fraction of the blood sampled. Prior art FC systems dilute a blood sample of approximately 200μl with a large volume of reagents and then analyze a small fraction of that diluted material. Because the amount of material actually examined by these prior art instruments is a function of several potentially inaccurate dilution and metering steps, quantitation (the number of cells/μl) is determined by calibrating the instrument with samples having known constituents and then using these calibration values as constants to calculate the results of actual samples. As can be appreciated, if any of the many measurement or dilution steps drifts, the accuracy of the instrument will be compromised. This potential for drift and subsequent error requires that control material be frequently run, which increases the cost and reduces the operational efficiency of the operation of these prior art instruments. The

previously referenced method described in U.S. Patent Publication No. 20020055178 also does not measure the entire aliquoted sample but instead uses a field-by-field volume calibration to obviate the need for instrument calibration.

[0013] This invention, in contrast to all prior art, examines the entirety of a tiny sample of undiluted blood present in a thin film confined between two relatively planar substrates, at least one of which is transparent, and whose minimum separation is controlled by a plethora of separator elements. All cellular elements therewithin are enumerated, in contrast to all other methods, where only a portion of the sample is examined.

[0014] This invention is performed by depositing a tiny sample of blood from a sample tube or other container onto a substrate such as a Mylar film. When a second film is brought into contact with the first, the deposited sample spreads by capillarity until a monolayer of blood is formed. Because the capillary forces are so strong, a means of providing a minimum separation between the layers is provided to prevent the cells being ruptured by the compression resulting from the capillary forces. It is an important characteristic of this type of capillary spread that the larger cellular elements, particularly the red blood cells, will flow faster than the plasma, thus concentrating the majority of the red blood cells in the periphery of the film as a contiguous sheet, with the center relatively sparsely populated with individual red blood cells, which can be easily identified and separately examined. The smaller elements, such as platelets, remain relatively uniformly distributed within the plasma fraction and can also be easily enumerated. The white blood cells, because they are larger than the red blood cells and preferably of larger diameter than the minimum film thickness, are isolated and slightly compressed, which offers an optimal image for individual cell classification and viewing. Because all cellular elements within the film are enumerated, the non-uniform distribution does not affect the accuracy of the cell counts but is used to advantage in the analysis.

[0015] This method requires that the exact amount of deposited blood be known. Although it is possible to deposit a specific amount of blood on the substrate, it is preferable to deposit an approximate amount and indirectly measure the volume. This can be done by one of three mechanisms; the volume of the drop of blood when first deposited can be calculated by interferometric imaging using optical techniques available from sources such as the Zygo Corporation, the volume of blood following

film formation is calculated by measuring the area of the film and multiplying this by the average height of the film as determined by the separator features, or tiny droplets of non-dispersing dye are added to at least on of the film surfaces before approximating them, and the OD of the resultant sandwiched droplet is then used to indirectly measure the thickness.

[0016] The principle of this invention requires that at some time in the analysis, the average blood film thickness be known, and that this thickness be relatively uniform, such that if measurements are carried out in, for example, ten randomly chosen microscopic fields of approximately 0.25 square millimeters in area, the average thickness of these ten areas as compared to the average area of the entire film will be within less than plus or minus five percent. This spacing is accomplished by the separator features, which are preferably beads of known and precisely controlled diameter of preferably about 5μ in diameter, which are available from a number of sources, such as Bangs Laboratory. These beads are randomly distributed on at least one of the substrates and are preferably attached as part of the reagent film containing the staining material. The material retaining the beads should be such that they remain affixed to the substrate until at least after blood film movement has ceased so that they will not be swept away. The number of beads is preferably at least one per examined field but may be many times that number without adversely affecting the results. An alternate means of providing the separator features is to negatively emboss one of the substrates with projections having approximately the same height of about 5μ, for example by laser-etching pits in a nip-roller and passing one substrate film through said nip-roller assembly.

[0017] The principles of the invention are further clarified by referring to the following figures, where:

[0018] Figure 1 is a schematic of the reading section on an instrument having a disposable cassette containing reels of the substrates; and

[0019] Figure 2 is a schematic of the cassette immediately after a drop of blood has been applied to a substrate; and

[0020] Figure 3 is a schematic of the cassette as the substrates are joined, forming the blood film; and

[0021] Figure 4 is a macroscopic schematic view of a typical blood film; and

[0022] Figure 5 is a microscopic view from the central region 'c' of said film; and

[0023] Figure 6 is a microscopic view from peripheral region 'p' of said film.

[0024] Figure 1 shows an instrument generally denoted as 2 containing a cassette 3 in which are disposed reels of lower substrate tape 4, upper substrate tape 5, and take-up reel 6. Advancement of the tapes is controlled by take-up nip-rollers 7, which apply traction to the combined tapes at a point remote from the examination area 8 and can act to draw the substrate tapes 4 and 5 from their reels as required. Optical analysis system 9, which consists of joined components including a lens 10, a variable-wavelength light source 11 and a CCD camera 12 are movable in three dimensions so as to allow the optical system 9 to focus upon the joined tapes in the film area 8 and provide X-Y movement so as to allow scanning of the entire film area, all under control of the system computer 13. Not shown is the sampling probe for extracting blood from the sample tube and depositing a small drop on the lower substrate film. This sampling device can take the form of a tube-piercing or similar probe, which uses a stepping motor-driven syringe to extract and deposit blood samples. These devices are widely employed and well known to the art.

[0025] Figure 2 shows the assembly of Figure 1 just after a drop of blood 14 has been deposited onto lower substrate tape 4, for example a Mylar film of approximately 0.005 inches (140μ) thickness, such as that used for photographic film.

[0026] In Figure 3, the nip-roller assembly 7, which is moved by a stepper motor under control of the instrument's computer, has advanced the tapes to a point just past rollers 7, where the tapes 4 and 5 are opposed to the point where they just touch, causing the blood to contact the upper tape 5 and thus spread to form film 16. The area of the tapes containing the film is then advanced so as to be readable by optical assembly 9.

[0027] Figure 4 is a schematic view of the entire area of the formed blood film 16, which generally has an irregular border. Because of the unique flow characteristics of blood, the cellular components are highly concentrated at the periphery as in the representative area 'p' and are relatively sparse in the central area 'c'. It is this self-segregation of the sample components, which allows the operation of the instant invention. Once the blood film has formed, its analysis proceeds in several steps:

[0028] The central region of the film 'c' is examined first so as to obtain the mean

cellular hemoglobin (MCH) measurement and information to be used in determining the platelet count. Figure 5 shows a representative high-power magnification of a field from that region, where individual red blood cells 17 are found, along with the platelets 18 and occasional white blood cells 19. The separator features, in this case uniform beads 20, are also seen.

[0029] The MCH is measured by calculating the hemoglobin content in a representative number of individual red cells and averaging them. This examination is preferably adaptive, where the running standard deviation of the result is used to determine how many cells must be examined before an average of acceptable accuracy is obtained. For normal samples this may be as few as 10 cells. Abnormal samples, on the other hand, may require the analysis of 100 cells. The calculation of the cellular hemoglobin for each cell is performed by obtaining the integral optical density (OD) of each cell at wavelengths characteristic of hemoglobin, such as 413nm or 540nm, and converting this integral OD to a hemoglobin value by multiplying it by published constants for those wavelengths. At the same time, the platelet count/plasma volume is determined by identifying and enumerating the platelets in fields, or portions of fields consisting solely of plasma. Since the thickness of the film is known, being a function of the separator features, averaging a number of said fields gives an accurate figure for the platelet count/plasma volume, which will be later corrected using the hematocrit to obtain a platelet count/blood volume. White blood cells 19 are counted and enumerated as they are located.

[0030] The peripheral region of the film 'p' is next examined. Figure 6 shows a representative high-power view of this region, where the red blood cells 17 are tiled in formations where the cell membranes are closely approximated with essentially no plasma between them. Widely separated white blood cells 19 are also seen here, but relatively few platelets 18 are found.

[0031] The mean cell volume (MCV) is determined by averaging several fields or portions of fields containing only red blood cells. In such a field, the number of red blood cells can be determined in one of two methods. The hemoglobin in the entire field can be determined by integral OD measurements described above, and this value, divided by the MCH as determined above, gives the number of red blood cells in that field. The volume within the observed field is then calculated by multiplying the observed area by the average film height, and this volume, divided by the number

of the red blood cells calculated to be in the observed area gives the MCV. Another method uses standard edge-finding image processing to identify the fine lines between the individual red blood cells in an observed field, enumerate them and perform the MCV calculation as described.

[0032] When the entire film has been examined, the total area is calculated as the sum of all non-overlapping fields, and this area, multiplied by the average film thickness, is the total volume of blood in the film. The total red blood cell count is therefore the sum of all of the red blood cells in each field divided by the volume of the blood film. Similarly, the white blood cell count is the sum of all of the white blood cells in each field divided by the volume of the blood film. The hematocrit, which is the percentage of the total volume occupied by the red blood cells, is determined by multiplying the total red blood cell count by the MCV as determined previously. The total platelet count is the average number of platelets per plasma volume divided by the hematocrit.

[0033] The characterization of the white blood cells (white blood cell differential count) is performed by the classification of each individual white blood cell as it is encountered using either traditional image-processing methods or by the technique described in U.S. Patent Nos. 5,321,975 and 6,350,613. The stain, preferably Astrozone Orange differentially colors the different classes of white blood cells as has been described in U.S. Patent No. 6,235,536. Because the white blood cells are slightly compressed and readily imaged, stored images of cells are viewable by the technologist in the case of questionable cell classifications.

[0034] The reagent and separator coated tape substrate can be formed by a continuous process, where a slurry of the stain, beads, and a binder, such as polyvinylpyrollodine are coated onto one side of the tape by rolling or immersion bath. The tape is dried and then run through a polished nip-roller, where the separator particles are forced into an average height by using the "highest-nail" principle. The nip-roller pressure is empirically set so that all beads would be slightly compressed, but if a bead of larger diameter is encountered, it is subjected to high pressure, which deforms it toward the average. The particular batch of tape in each disposable cassette is then calibrated by creating a thin film using a dye of known optical density. The average optical density of the film therefore represents the average height of a formed film, and this calibration information can be incorporated into a label on the

cassette to be used by the instrument.

[0035] It can be appreciated that this invention can also perform most of the functions of a flow-cytometer by adding fluorescent or other markers to cell-specific ligands and examining the film to determine which cells have the ligand-marker bound to their surfaces.

Claims

1. An apparatus for forming uniform films of a biologic fluid, comprising:

a top layer;

a bottom layer, of which at least one is transparent; and

a separation means spacing the two said layers, where at least one of the top layer, bottom layer, or separator means is deformable to the extent that the deformable component allows the separation of the said layers to have a spacing of an accurate average dimension, regardless of small variations in the size of individual separator means.

2. The apparatus of claim 1 wherein the layers are formed from reels of flexible plastic.

3. The apparatus of claim 1 wherein one layer is formed from linked rigid elements and the other is formed from a reel of flexible plastic.

4. The apparatus of claim 1 wherein the separator means include uniformly dyed, slightly compressible plastic beads, and the height of the spacing created is calculated by measuring the average attenuation of light transmitted through said particles.

5. The apparatus of claim 1 where the separator means are projections of uniform height attached to or manufactured integrally with at least one of said layers.

6. A method of enumerating the cellular or particulate constituents of a sample of whole, anticoagulated blood, comprising the steps of:

placing a quantity of blood onto one surface of the apparatus of claim 1;

approximating the two surfaces to form a film of blood confined between the two layers as separated by the separator means;

determining the volume of blood contained within the film;

directly or indirectly enumerating all constituents of interest within substantially the entire area of the film; and

expressing the enumerated constituents as a count per unit volume.

7. The method of claim 6 wherein the film volume is calculated by multiplying the average height of the film times its measured area.

8. The method of claim 6 wherein the film volume is calculated by interferometric imaging of the drop of blood added to the layer surface prior to approximating the two surfaces.

Application Data Sheet

Application Information

Application number::

Filing Date:: 04/07/04

Application Type:: provisional

Subject Matter:: utility

Suggested classification::

Suggested Group Art Unit::

CD-ROM or CD-R?:: no

Number of CD disks::

Number of copies of CDs::

Sequence submission?:: no

Computer Readable Form (CRF)?:: no

Title:: Apparatus and Method for Performing Complete Blood Counts

Attorney Docket Number:: 5169-0016

Request for Early Publication?:: n.a.

Request for Non-Publication?:: n.a.

Suggested Drawing Figure:: n.a.

Total Drawing Sheets:: 3

Small Entity?:: yes

Latin name::

Variety denomination name::

Petition included?:: no

Petition type::

Licensed US Govt. Agency::

Contract or Grant numbers::

Secrecy Order in Parent Appl.?:: no

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Applicant Authority Type:: inventor

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Status:: full capacity

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Associate	44,322	Nicholas J. Tuccillo
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Associate	34,988	Andrea C. Walsh

Domestic Priority Information

Application::	Continuity Type::	Parent Application::	Parent Filing Date::

Foreign Priority Information

Country::	Application number::	Filing Date::	Priority Claimed:

Assignee Information

Assignee name:: n.a.

Street of mailing address::

City of mailing address::

State or Province of mailing address::

Country of mailing address::

Postal or Zip code of mailing address::

Figure 1

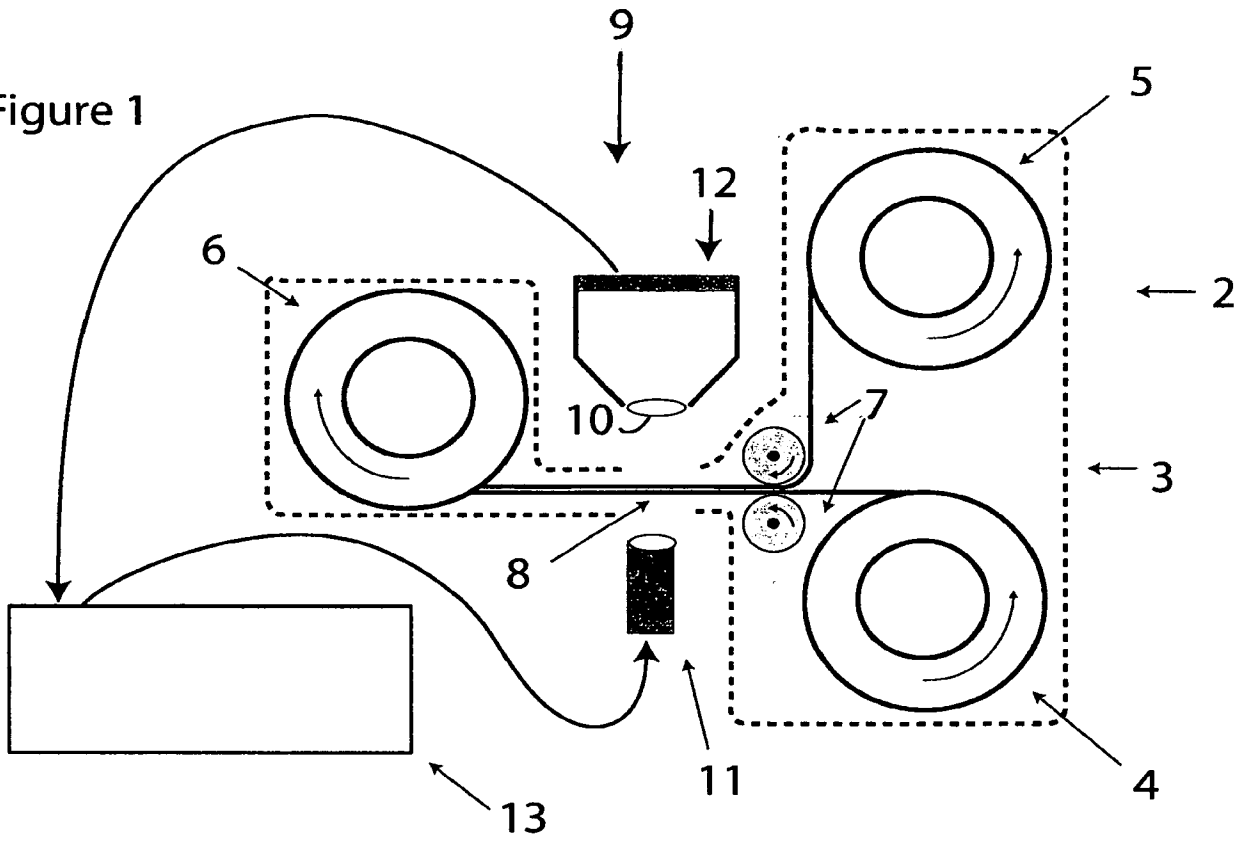

Figure 2

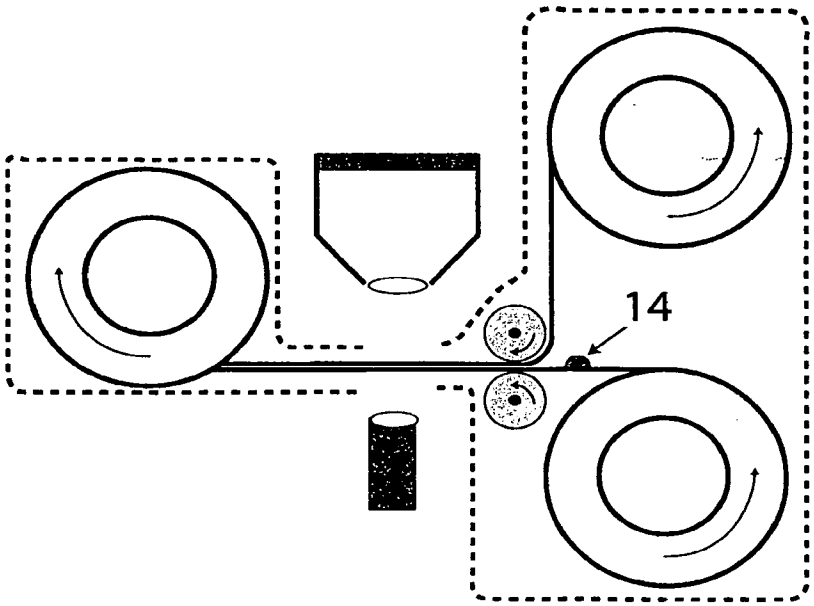

Figure 3

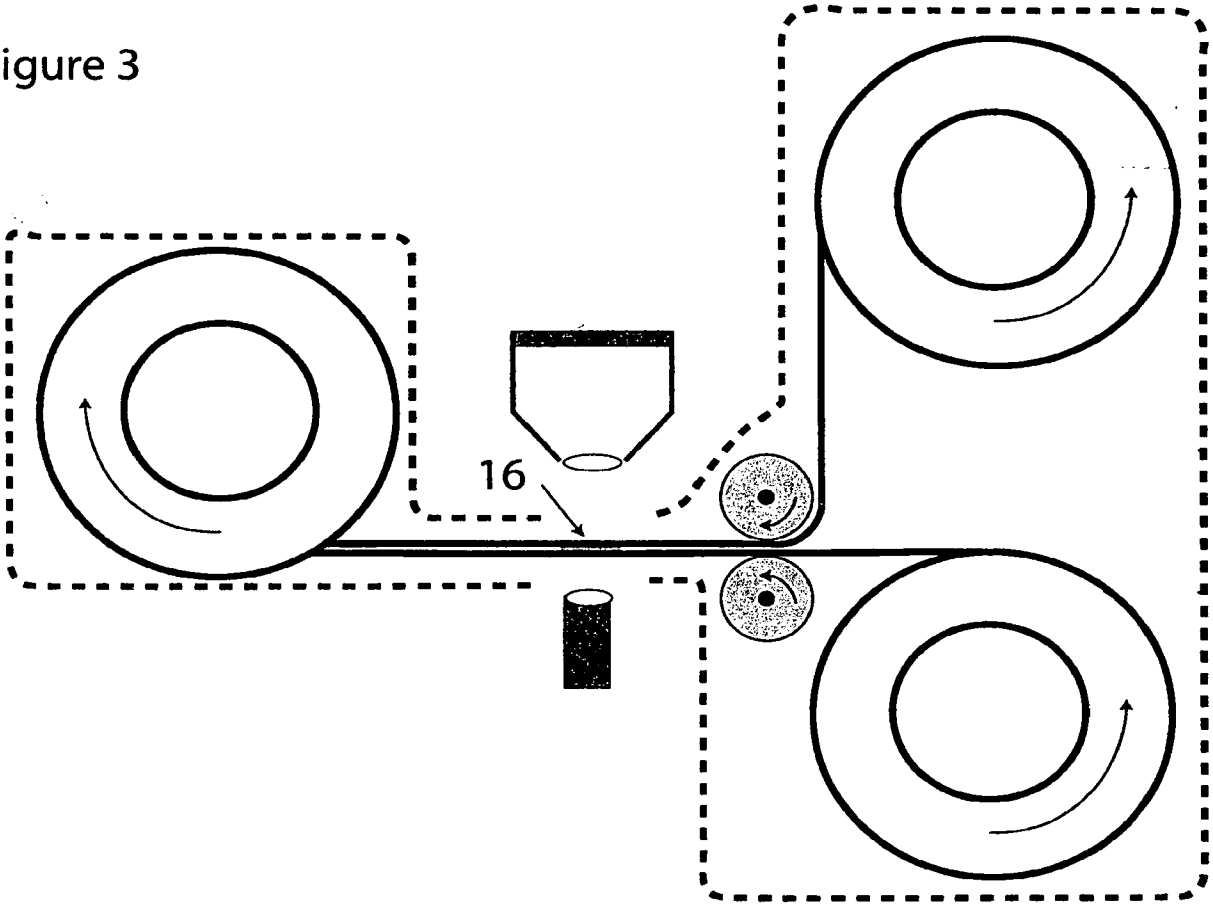

Figure 4

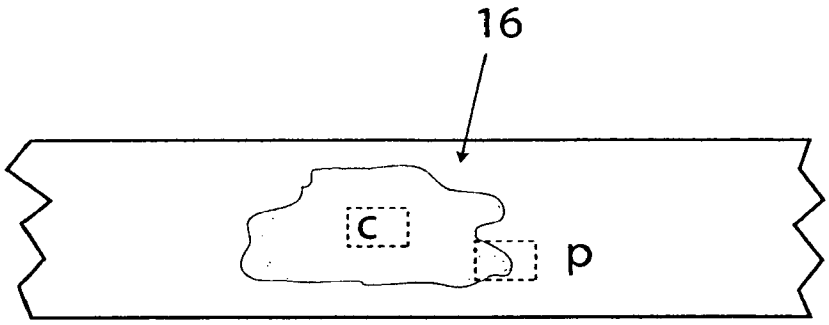

Figure 5

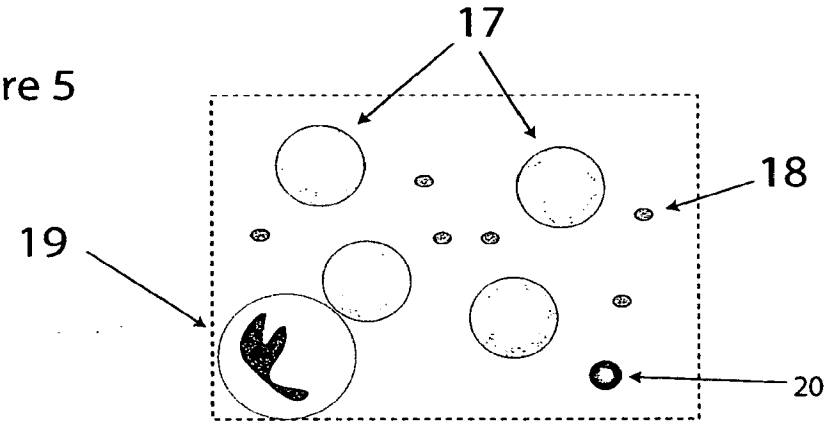

Figure 6